

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a flavonoid 3', 5' hydroxylase (F3'5'H) or a polypeptide having F3'5'H activity wherein expression of said nucleic acid molecule in a rose petal tissue results in detectable levels of *delphinidin* or *delphinidin-based molecules* as measured by a chromatographic technique.
2. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a F3'5'H or a polypeptide having F3'5'H activity wherein expression of said nucleic acid molecule in a rose petal tissue results in a sufficient level and length of transcript which is translated to said F3'5'H as determined by detectable levels of *delphinidin* or *delphinidin-based molecules* as measured by a chromatographic technique.
3. The isolated nucleic acid molecule of claim 1 or 2 wherein expression of said nucleic acid molecule in said rose petal results in a visually detectable colour change.
4. The isolated nucleic acid molecule of any one of claims 1 to 3, wherein the nucleic acid molecule is derived from a plant selected from the list comprising a *Viola* spp., *Salvia* spp., *Sollya* spp., *Lavandula* spp. and *Kennedia* spp.
5. The isolated nucleic acid molecule of claim 4, wherein the nucleic acid molecule is derived from a *Viola* spp. plant.
6. The isolated nucleic acid molecule of claim 5, wherein the nucleic acid molecule is derived from the *Viola* spp., cultivar Black Pansy.

- 135 -

7. The isolated nucleic acid molecule of any one of claims 5 or 6, wherein the nucleotide sequence encodes a F3'5'H comprising an amino acid sequence selected from SEQ ID NO:10, SEQ ID NO:12, an amino acid sequence having at least about 40% similarity to SEQ ID NO:10 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:12.

8. The isolated nucleic acid molecule of claim 7, comprising a nucleotide sequence selected from SEQ ID NO:9, SEQ ID NO:11, a nucleotide sequence having at least about 40% identity to SEQ ID NO:9, a nucleotide sequence having at least about 40% identity to SEQ ID NO:11, a nucleotide sequence capable of hybridizing to SEQ ID NO:9 or its complement under low stringency conditions and a nucleotide sequence capable of hybridizing to SEQ ID NO:11 or its complement under low stringency conditions.

9. The isolated nucleic acid molecule of claim 8, comprising the nucleotide sequence set forth in SEQ ID NO:9.

10. The isolated nucleic acid molecule of claim 8, comprising the nucleotide sequence set forth in SEQ ID NO:11.

11. The isolated nucleic acid molecule of claim 4, wherein the nucleic acid molecule is derived from *Salvia spp.*.

12. The isolated nucleic acid molecule of claim 11, wherein the nucleotide sequence encodes a F3'5'H comprising an amino acid sequence selected from SEQ ID NO:14, SEQ ID NO:16, an amino acid sequence having at least about 40% similarity to SEQ ID NO:14 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:16.

- 136 -

13. The isolated nucleic acid molecule of claim 12, comprising a nucleotide sequence selected from SEQ ID NO:13, SEQ ID NO:15, a nucleotide sequence having at least about 40% identity to SEQ ID NO:13, a nucleotide sequence having at least about 40% identity to SEQ ID NO:15, a nucleotide sequence capable of hybridizing to SEQ ID NO:13 or its complement under low stringency conditions and a nucleotide sequence capable of hybridizing to SEQ ID NO:15 or its complement under low stringency conditions.

14. The isolated nucleic acid molecule of claim 13, comprising the nucleotide sequence set forth in SEQ ID NO:13.

15. The isolated nucleic acid molecule of claim 13, comprising the nucleotide sequence set forth in SEQ ID NO:15.

16. The isolated nucleic acid molecule of claim 4, wherein the nucleic acid molecule is derived from *Sollya spp.*

17. The isolated nucleic acid molecule of claim 16, wherein the nucleotide sequence encodes a F3'5'H comprising an amino acid sequence selected from SEQ ID NO:18 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:18.

18. The isolated nucleic acid molecule of claim 17, comprising a nucleotide sequence selected from SEQ ID NO:17, a nucleotide sequence having at least about 40% identity to SEQ ID NO:17 and a nucleotide sequence capable of hybridizing to SEQ ID NO:17 or its complement under low stringency conditions.

19. The isolated nucleic acid molecule of claim 18, comprising the nucleotide sequence set forth in SEQ ID NO:17.

20. The isolated nucleic acid molecule of claim 4, wherein the nucleic acid molecule is derived from *Kennedia spp.*

- 137 -

21. The isolated nucleic acid molecule of claim 20, wherein the nucleotide sequence encodes a F3'5'H comprising an amino acid sequence selecting from SEQ ID NO:27 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:27.

22. The isolated nucleic acid molecule of claim 21, comprising a nucleotide sequence selected from SEQ ID NO:26, a nucleotide sequence having at least about 40% identity to SEQ ID NO:26 and a nucleotide sequence capable of hybridizing to SEQ ID NO:26 or its complement under low stringency conditions.

23. The isolated nucleic acid molecule of claim 22, comprising the nucleotide sequence set forth in SEQ ID NO:26.

24. The isolated nucleic acid molecule of claim 4, wherein the nucleic acid molecule is derived from *Lavandula spp.*

25. The isolated nucleic acid molecule of claim 24, wherein the nucleotide sequence encodes a F3'5'H comprising an amino acid sequence selected from SEQ ID NO:32 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:32.

26. The isolated nucleic acid molecule of claim 25, comprising a nucleotide sequence selected from SEQ ID NO:31, a nucleotide sequence having at least about 40% identity to SEQ ID NO:31 and a nucleotide sequence capable of hybridizing to SEQ ID NO:31 or its complement under low stringency conditions.

27. The isolated nucleic acid molecule of claim 26, comprising the nucleotide sequence set forth in SEQ ID NO:31.

- 138 -

28. The isolated nucleic acid molecule of any one of claims 1- to 4, wherein the nucleotide sequence comprises an overall percentage of less than or equal to 54% of the nucleotides

- (i) A, or
- (ii) T, or
- (iii) A and T

in the third nucleotide position of each codon.

29. A construct comprising a sequence of nucleotides comprising:

- (i) a promoter which is operable in rose petal tissue and wherein said promoter is operably linked to,
- (ii) a nucleic acid molecule encoding F3'5'H, or complementary to a sequence encoding a F3'5'H or a polypeptide having F3'5'H activity wherein expression of said nucleic acid molecule in a rose petal tissue results in detectable levels of *delphinidin* or *delphinidin-based molecules* as measured by a chromatographic technique and wherein said nucleic acid molecule is derived from a plant selected from the group consisting of a *Viola spp.*, *Salvia spp.*, *Solvia spp.*, *Lavandula spp.* and *Kennedia spp.*

30. A construct comprising a sequence of nucleotides comprising:

- (i) a promoter which is operable in rose petal tissue and wherein said promoter is operably linked to,
- (ii) a nucleic acid molecule encoding F3'5'H, or complementary to a sequence encoding a F3'5'H or a polypeptide having F3'5'H activity wherein expression of said nucleic acid molecule in a rose petal tissue results in a sufficient level and length of transcript which is translated to said F3'5'H as determined by detectable levels of *delphinidin* or *delphinidin-based molecules* as measured by a chromatographic technique.

- 139 -

31. The construct of claim 29 or 30, wherein expression of said construct in said rose petal results in a visually detectable colour change.

32. The construct of any one of claims 29 to 31, wherein said promoter is selected from the group consisting of rose CHS, chrysanthemum CHS and CaMV 35S.

33. A construct of any one of claims 29 to 31 wherein said promoter comprises SEQ ID NO:5, or a functional equivalent thereof.

34. A construct of any one of claims 29 to 31 wherein said promoter comprises SEQ ID NO:30, or a functional equivalent thereof.

35. The construct of any one of claims 29 to 34, wherein the nucleic acid molecule is derived from a *Viola spp.*

36. The isolated nucleic acid molecule of claim 35, wherein the nucleotide sequence encodes F3'5'H comprising an amino acid sequence selected from SEQ ID NO:10, SEQ ID NO:12, an amino acid sequence having at least about 40% similarity to SEQ ID NO:10 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:12.

37. The isolated nucleic acid molecule of claim 36, comprising a nucleotide sequence selected from SEQ ID NO:9, SEQ ID NO:11, a nucleotide sequence having at least about 40% identity to SEQ ID NO:9, a nucleotide sequence having at least about 40% identity to SEQ ID NO:11, a nucleotide sequence capable of hybridizing to SEQ ID NO:9 or its complement under low stringent conditions and a nucleotide sequence capable of hybridizing to SEQ ID NO:11 or its complement under low stringent conditions.

38. The isolated nucleic acid molecule of claim 37, wherein the gene comprises the nucleotide sequence set forth in SEQ ID NO:9.

- 140 -

39. The isolated nucleic acid molecule of claim 37, wherein the gene comprises the nucleotide sequence set forth in SEQ ID NO:11.

40. The isolated nucleic acid molecule of any one of claims 29 to 34, wherein the nucleic acid molecule is derived from *Salvia spp.*

41. The isolated nucleic acid molecule of claim 40, wherein the gene comprises a nucleotide sequence encoding F3'5'H comprising an amino acid sequence selected from SEQ ID NO:14, SEQ ID NO:16, an amino acid sequence having at least about 40% similarity to SEQ ID NO:14 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:16.

42. The isolated nucleic acid molecule of claim 41, wherein the gene comprises a nucleotide sequence selected from SEQ ID NO:13, SEQ ID NO:15, a nucleotide sequence having at least about 40% identity to SEQ ID NO:13, a nucleic sequence having at least about 40% identity to SEQ ID NO:15, a nucleotide sequence capable of hybridizing to SEQ ID NO:13 or its complements under low stringent conditions and a nucleotide sequence capable of hybridizing to SEQ ID NO:15 or is complement under low stringent conditions.

43. The isolated nucleic acid molecule of claim 42, wherein the gene comprises the nucleotide sequence set forth in SEQ ID NO:13.

44. The isolated nucleic acid molecule of claim 42, wherein the gene comprises the nucleotide sequence set forth in SEQ ID NO:15.

45. The isolated nucleic acid molecule of any one of claims 29 to 34, wherein the gene is derived from *Sollya spp.*

- 141 -

46. The isolated nucleic acid molecule of claim 45, wherein the gene encodes a F3'5'H comprising an amino acid sequence selected from SEQ ID NO:18 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:18.

47. The isolated nucleic acid molecule of claim 46, wherein the gene comprises a nucleotide sequence selected from SEQ ID NO:17, a nucleotide sequence having at least about 40% identity to SEQ ID NO:17 and a nucleotide sequence capable of hybridizing to SEQ ID NO:17 or its complements under low stringent.

48. The isolated nucleic acid molecule of claim 47, wherein the gene comprises the nucleotide sequence set forth in SEQ ID NO:17.

49. The isolated nucleic acid molecule of any one of claims 29 to 34, wherein the gene is derived from *Kennedia spp.*

50. The isolated nucleic acid molecule of claim 49, wherein the gene encodes F3'5'H comprising an amino acid sequence selected from SEQ ID NO:27 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:27.

51. The isolated nucleic acid molecule of claim 50, wherein the gene comprises a nucleotide sequence selected from SEQ ID NO:26, a nucleotide sequence having at least about 40% identity to SEQ ID NO:26 and a nucleotide sequence capable of hybridizing to SEQ ID NO:26 or its complements under low stringent.

52. The isolated nucleic acid molecule of claim 51, wherein the gene comprises the nucleotide sequence set forth in SEQ ID NO:26.

53. The isolated nucleic acid molecule of any one of claims 29 to 34, wherein the gene is derived from *Lavandula spp.*

- 142 -

54. The isolated nucleic acid molecule of claim 53, wherein the gene encodes *F3'5'H* comprising an amino acid sequence selected from SEQ ID NO:32 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:32.

55. The isolated nucleic acid molecule of claim 54, wherein the gene comprises a nucleotide sequence selected from SEQ ID NO:31 and a nucleotide sequence having at least about 40% identity to SEQ ID NO:31, a nucleotide sequence capable of hybridizing to SEQ ID NO:31 or its complements under low stringent conditions.

56. The isolated nucleic acid molecule of claim 55, wherein the gene comprises the nucleotide sequence set forth in SEQ ID NO:31.

57. A method for producing a transgenic flowering plant capable of synthesizing a *F3'5'H*, said method comprising stably transforming a cell of a suitable plant with a nucleic acid sequence as defined in any one of claims 1 to 28, under conditions permitting the eventual expression of said nucleic acid sequence, regenerating a transgenic plant from the cell and growing said transgenic plant for a time and under conditions sufficient to permit the expression of the nucleic acid sequence.

58. A method for producing a transgenic plant with reduced indigenous or existing *F3'5'H* activity, said method comprising stably transforming a cell of a suitable plant with a nucleic acid molecule as defined in any one of claims 1 to 28, regenerating a transgenic plant from the cell and where necessary growing said transgenic plant under conditions sufficient to permit the expression of the nucleic acid.

59. A method for producing a genetically modified plant with reduced indigenous or existing *F3'5'H* activity, said method comprising altering the *F3'5'H* gene through modification of the indigenous sequences via homologous recombination from an appropriately altered *F3'5'H* gene as defined in any one of claims 1 to 28, or a derivative or part thereof introduced into the plant cell, and regenerating the genetically modified plant from the cell.

- 143 -

60. A method for producing a transgenic flowering plant exhibiting altered inflorescence properties, said method comprising stably transforming a cell of a suitable plant with a nucleic acid sequence as defined in any one of claims 1 to 28, regenerating a transgenic plant from the cell and growing said transgenic plant for a time and under conditions sufficient to permit the expression of the nucleic acid sequence.

61. A method for producing a flowering plant exhibiting altered inflorescence properties, said method comprising alteration of a *F3'5'H* gene as defined in any one of claims 1 to 28, through modification of the indigenous sequences *via* homologous recombination from an appropriately altered *F3'5'H* gene or derivative or part thereof introduced into the plant cell, and regenerating the genetically modified plant from the cell.

62. A method for producing a transgenic plant capable of expressing a recombinant gene encoding *F3'5'H* as defined in any one of claims 1 to 28, or part thereof or which carries a nucleic acid sequence which is substantially complementary to all or a part of an mRNA molecule encoding said *F3'5'H*, said method comprising stably transforming a cell of a suitable plant with the isolated nucleic acid molecule comprising a sequence of nucleotides encoding, or complementary to a sequence encoding *F3'5'H*, where necessary under conditions permitting the eventual expression of said isolated nucleic acid molecule, and regenerating a transgenic plant from the cell.

63. A genetically modified plant or part thereof or cells therefrom comprising an isolated nucleic acid molecule of any one of claims 1 to 28.

64. A genetically modified plant or part thereof or cells therefrom comprising an isolated nucleic acid molecule of any one of claims 1 to 28 or comprising a reduced level of expression of a nucleic acid molecule of any one of claims 1 to 28.

- 144 -

65. A genetically modified plant or part thereof or cells therefrom comprising an isolated nucleic acid molecule of any one of claims 1 to 28 or comprising an increased level of expression of a nucleic acid molecule of any one of claims 1 to 28.

66. The genetically modified plant or part thereof or cells therefrom any one of claims 63 to 65, wherein the plant part is selected from sepal, bract, petiole, peduncle, ovaries, anthers, flowers, fruits, nuts, roots, stems, leaves, seeds.

67. The genetically modified plant or part thereof or cells therefrom of any one of claims 63 to 66, wherein the plant is a horticultural species, agricultural species or ornamental species.

68. Use of an isolated nucleic acid molecule as defined in any one of claims 1 to 28, in the manufacture of a genetic construct capable of expressing F3'5'H or down-regulating an indigenous F3'5'H enzyme in a plant.

69. A gene silencing construct comprising an isolated nucleic acid molecule as defined in any one of claims 1 to 28 or a complex thereof.

70. The genetically modified plant or part thereof or cells therefrom of 63 to 66, wherein the plant is selected from a rose, carnation, lisianthus, petunia, lily, pansy, gerbera, chrysanthemum, geranium, *Torenia*, *Begonia*, *Cyclamen*, *Nierembergia*, *Catharanthus*, *Pelargonium*, orchid, grape, apple, *Euphorbia* or *Fuchsia*.

71. An extract from a genetically modified plant or part thereof or cells therefrom from any one of claims 63 to 67 and 70.

72. The extract of claim 71, wherein the extract is a flavouring or food additive or health product or beverage or juice or colouring.

- 145 -

73. The method of any one of claims 57 to 62 wherein the genetically modified plant or part thereof or cells therefrom exhibit altered inflorescence.

74. An isolated recombinant F3'5'H or peptide having F3'5'H activity encoded by a nucleic acid molecule as defined in any one of claims 1 to 28.

75. The isolated recombinant F3'5'H or peptide having F3'5'H activity of claim 74, wherein the recombinant F3'5'H or peptide having F3'5'H activity is a fusion molecule comprising two or more heterologous amino acid sequences.

76. An isolated recombinant F3'5'H or peptide having F3'5'H activity nucleic acid molecule of any one of claims 1 to 28 comprising a fusion of two or more heterologous nucleotide sequences.

77. A prokaryotic organism carrying a genetic sequence encoding a F3'5'H molecule according to any one of claims 1 to 28 extrachromasomally in plasmid form.

78. A eukaryotic organism carrying a genetic sequence encoding a F3'5'H molecule according to any one of claims 1 to 28 extrachromasomally in plasmid form.

79. The use of a nucleic acid molecule of any one of claims 1 to 28 in the manufacture of a genetically modified plant or part thereof or cells therefrom.

80. The genetically modified plant or part thereof or cells therefrom of claim 79, wherein the genetically modified plant or part thereof or cells therefrom exhibit altered flowers or inflorescence.

81. The use of a nucleic acid sequence as defined in any one of claims 1 to 28 in the manufacture of a genetic construct capable of expressing F3'5'H or down-regulating an indigenous F3'5'H enzyme in a plant.

- 146 -

82. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a F3'5'H or a polypeptide having F3'5'H activity, wherein said nucleic acid molecule is derived from butterfly pea.

83. The isolated nucleic acid molecule of claim 81, wherein the nucleotide sequence encodes a F3'5'H comprising an amino acid sequence selecting for SEQ ID NO:21 and an amino acid sequence having at least about 40% similarity to SEQ ID NO:21.

84. The isolated nucleic acid molecule of claim 83, comprising a nucleotide sequence selected from SEQ ID NO:20, a nucleotide sequence having at least about 40% identity to SEQ ID NO:20 and a nucleotide sequence capable of hybridizing to SEQ ID NO:20 or its complement under low stringency conditions.

85. The isolated nucleic acid molecule of claim 84, comprising the nucleotide sequence set forth in SEQ ID NO:20.

86. The isolated nucleic acid molecule of any one of claims 1 to 4, wherein the nucleotide sequence comprises an overall percentage of less than or equal to 55% of the nucleotides

- (i) A, or
- (ii) T, or
- (iii) A and T.

87. An isolated nucleic acid molecule comprising SEQ ID NO:5 or a functional equivalent thereof.

88. An isolated nucleic acid molecule comprising SEQ ID NO:30 or a functional equivalent thereof.

- 147 -

89. An isolated nucleic acid molecule which has been modified so as to comprise a sequence of nucleotides encoding or complementary to a sequence encoding F3'5'H or a polypeptide having F3'5'H activity wherein expression of said nucleic acid molecule in a rose petal tissue results in detectable levels of *delphinidin or delphinidin-based molecules* as measured by a chromatographic technique wherein the nucleic acid molecule is derived from a plant selected from the list comprising *Petunia spp.*, *Gentiana spp.* and *Clitoria spp.*

90. An isolated nucleic acid molecule which has been modified so as to comprise a sequence of nucleotides encoding or complementary to a sequence encoding F3'5'H or a polypeptide having F3'5'H activity wherein expression of said nucleic acid molecule in a rose petal tissue results in a sufficient level and length of transcript which is translated to said F3'5'H as determined by detectable levels of *delphinidin or delphinidin-based molecules* as measured by a chromatographic technique wherein the nucleic acid molecule is derived from a plant selected from the list comprising petunia, gentiana and butterfly pea.

91. The use of a nucleic acid sequence as defined in claim 89 or 90 in the manufacture of a genetic construct capable of expressing F3'5'H or down-regulating an indigenous F3'5'H enzyme in a plant.

92. The use of a nucleic acid sequence as defined in claim 89 or 90 in the manufacture of a genetically modified plant or part thereof or cells therefrom.